oligonucleotide, most conveniently via phosphoramidite chemistry. Convenient donors include FAM, HEX and TET.

We surprisingly found that, without the inclusion of a specific quencher, the tail alone can provide sufficient quenching of fluorescence. When the target binding region hybridises to a complementary sequence in the primer extension product a clear fluorescence signal is observed. The optimum point of attachment of the fluorophore may determined by routine experimentation. In a further aspect of the invention the signalling system comprises a fluorophore attached to the tail region of the primer, conveniently at or adjacent the termini 5' terminus of the primer. Whilst we do not wish to be limited by theoretical considerations, any G-rich sequence of at least 5 base pairs, such as at least 10 or at least 15, such as at least 20 base pairs may be used as a quencher species.

In a further specific embodiment, the primer tail includes an intercalating dye, hybridisation of the target binding region causes the dye to become incorporated between the bases of the double stranded DNA and thus to fluoresce. The dye should preferably have a low fluorescence when not intercalated, and a strong fluorescent enhancement upon intercalation. Again the preferred molecules should be easy to attach to the oligonucleotide by solid phase chemistry or by simple post-synthesis addition.

It will be appreciated that the overall length of the primer tail will be determined principally by the intended functions of its individual components. In general, the primer tail will be of at least 10 base pairs, such as at least 20, 30, 40 or 50 base pairs, for example 10-30 or 15-25 base pairs.

It is desirable that all dyes, quenchers, linkers/blockers should tolerate repeated rounds of PCR which include multiple exposures to high temperatures.

In a preferred aspect of the invention at least one component of the signalling system and the nucleic acid primer is an integral species.

The template nucleic acid is any convenient nucleic acid for analysis. Most commonly this will be DNA from an amplification reaction such as the PCR. This DNA target may have been derived from a reverse transcription (RT) reaction. Indeed, the primer of the invention may be used in the RT reaction itself and be used directly, without further amplification. Other in vitro amplification techniques such as ligase chain reaction (LCR), OLA, NASBA and Strand Displacement Amplification (SDA) may also be suitable. It is important however

that there is a single stranded intermediate which allows the target binding region to hybridise to a complementary sequence in the primer extension product. In general the method of our invention is used as the last (detection) step in the above methods. It will be appreciated that some optimisation/reconfiguration may be required but the relevant steps will be apparent to the artisan of ordinary skill.

Sources of sample nucleic acid include human cells such circulating blood, buccal epithelial cells, cultured cells and tumour cells. Also other mammalian tissue, blood and cultured cells are suitable sources of template nucleic acids. In addition, viruses, bacteriophage, bacteria, fungi and other micro-organisms can be the source of nucleic acid for analysis. The DNA may be genomic or it may be cloned in plasmids, bacteriophage, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs) or other vectors. RNA may be isolated directly from the relevant cells or it may be produced by *in vitro* priming from a suitable RNA promoter or by *in vitro* transcription.

The present invention may be used for the detection of variation in genomic DNA whether human, animal or other. It finds particular use in the analysis of inherited or acquired diseases or disorders. A particular use is in the detection of inherited disease. It will be appreciated that the target nucleic acid is directly or indirectly linked to the sequence or region of interest for analysis. In one preferred aspect the primer of the invention is used as the common primer in a PCR in combination with an ARMS primer (as disclosed in for example EP-B1-0 332 435). This is an example of indirect linkage to the sequence or region of interest. Alternatively the sequence or region of interest is identified when it interacts with the template specific region in an allele specific manner, preferably as an ARMS primer (see above). Alternatively, the sequence or region of interest may be identified by allele specific interaction with the target binding region in the primer tail. Still further, the sequence or region of interest may be a combination of the target region and template binding seqence in the primer provided that hybridisation of the target binding region in the primer tail is dependent on formation of a primer extension product.

In addition to the gene based diagnostics of human heritable disease, the invention will be useful in the detection of amplicons from other sources. A particular use is in the detection of infectious agents (bacteria, viruses etc), such as HIV, where the combination of allele specific priming and allelic discrimination via the target binding region offers opportunities to

monitor the emergence of particular variants of HIV within a virus population in a patient. Other infectious agents for which quantitative data (measured by Real time PCR) would be helpful include Hepatitis C virus and others.

In other medical microbiology applications it is important to be able to detect and quantify particular species of micro-organism. The use of fluorescent Scorpions primers greatly facilitates this.

The presence of bacteria in food or other products can also be usefully monitored using real time PCR with Scorpions fluorescence methods. The specificity of probe detection can be modified to permit or exclude the detection of related targets.

A particular advantage is that the novel primers of the invention need not be used at 100% primer concentration, that is to say the detection method works well even where only a small proportion of novel to conventional primer is used. Whilst we do not wish to be bound by theoretical considerations we believe that as little as a few percent, say up to 10%, up to 20%, such as up to 30% , up to 40% or up to 50% or 60% novel primer is used. Alternatively at least 50%, 60%, 70%, 80%, 90% or 100% novel primer is used.

The primer(s) can be added at any convenient stage in an amplification reaction, for example in the final amplification cycle, all that is required is one or more primer extension reactions. For homogeneous detection systems it is preferable to add the primer(s) at the start of any amplification procedure.

The primer tail may be configured in a number of different ways, the sole requirement is that the target binding region in the tail is available after primer extension to hybridise with a complementary sequence (if present) in the primer extension product. In its simplest form the primer tail is randomly coiled, if fluorescent detection means are used the primer is self-quenched prior to hybridisation of the target binding region.

The primer may include one or more regions of internal hybridisation which help stabilise the signalling system in a given position i.e. a particular configuration. Such region(s) are conveniently located within the primer tail and may each be of 2 or more base pairs. The configurations adopted are limited only by practical considerations and may include the use of one or more structures selected from hairpins, arms, elbows, stems, bubbles and loops. Once convenient structures have been devised these may be used as common features in the tailed primers of the invention.